

Supporting Information for:

Quantitation of IgG protein adsorption to gold nanoparticles using particle size measurement

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S.1

Derivation of Equation 2.

Starting from the basic equation for DCS:

$$t = \frac{\alpha}{(\rho_p - \rho_f)D_p^2}$$

With:

$$\alpha = \frac{18\eta \ln\left(\frac{R_f}{R_0}\right)}{\omega^2}$$

D_p is the total particle diameter, ρ_p is the total particle density, ρ_f is the fluid density and t is the sedimentation time between radii R_0 and R_f at angular frequency ω and fluid viscosity η . If a particle (D_0, ρ_0) acquires an shell of density ρ_s , the relationship between diameter and density is given by:

$$\frac{d\rho_p}{dD_p} = \frac{3(\rho_s - \rho_0)}{D_p}$$

The change in sedimentation time can be obtained from:

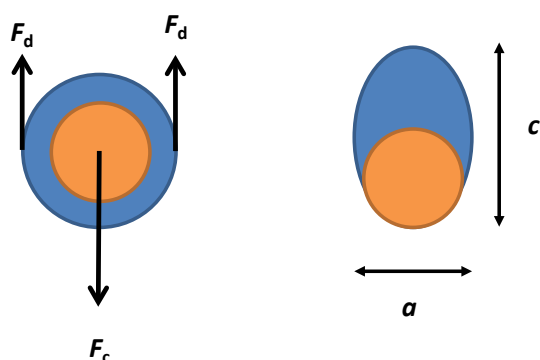
$$\frac{dt}{dD_p} = \frac{\partial t}{\partial D_p} + \left(\frac{\partial t}{\partial \rho_p} \frac{d\rho_p}{dD_p}\right) = \frac{-2\alpha}{(\rho_p - \rho_f)D_p^3} + \frac{-3\alpha(\rho_s - \rho_0)}{(\rho_p - \rho_f)^2 D_p^3} = \frac{-t}{D_p} \left(2 + 3 \frac{(\rho_s - \rho_0)}{(\rho_p - \rho_f)}\right)$$

Which, for small changes in diameter on a bare particle ($\rho_p = \rho_0, D_p = D_0$), may be approximated to:

$$\frac{\Delta t}{t} \approx \frac{\Delta D_p}{D_0} \left(3 \frac{(\rho_0 - \rho_s)}{(\rho_0 - \rho_f)} - 2\right) = \frac{2T}{D_0} \left(3 \frac{(\rho_0 - \rho_s)}{(\rho_0 - \rho_f)} - 2\right)$$

S.2

Redistribution of protein:



Shear stress on protein layer:

We consider that the protein shell is deformable and that in sedimentation the protein layer is deformed by the fact that the sedimentary force acts predominantly on the gold core, which would sediment faster without the protein shell, and the drag force acts on the outer layer of protein (for a sphere, the drag force is evenly distributed over the surface of the sphere).

The equatorial shear stress on the layer is given approximately by:

$$\frac{F_c + F_d}{A} \approx \frac{2F_c}{A} = \frac{2(\rho_0 - \rho_s) \frac{\pi}{6} D_0^3 R \omega^2}{\pi D_0^2} = \frac{(\rho_0 - \rho_s) R \omega^2}{3} D_0$$

Which, with $\rho_0 = 19300 \text{ kg m}^{-3}$, $\rho_s = 1000 \text{ kg m}^{-3}$, $R = 0.04 \text{ m}$, $\omega = 2094 \text{ rad s}^{-1}$ and $D_0 = 4 \times 10^{-8} \text{ m}$, provides an estimated shear stress of $\sim 40 \text{ Pa}$ for 40 nm gold particles at $20,000 \text{ RPM}$. The shear stress on other particles are estimated as $\sim 20 \text{ Pa}$ (20 nm at $20,000 \text{ RPM}$), $\sim 50 \text{ Pa}$ (60 nm at $18,000 \text{ RPM}$) and $\sim 40 \text{ Pa}$ (80 nm at $14,000 \text{ rpm}$). These stresses are comparable in magnitude to the shear moduli of soft hydrogels (10 Pa to 100 Pa), implying a significant elastic distortion of the adsorbed protein shell. For partial protein layers, a viscous response may also redistribute protein to the rear of the particle in a continual flow field.

Both the elastic and viscous response will cause the protein to accumulate behind the gold core and the shape may then be approximated as a prolate ellipsoid. The shape factors ($K = f/f_0$) for ellipsoids are well understood and can be calculated exactly (see, for example, A. J. Weinheimer, *Journal of the Atmospheric Sciences*, 1987, **44**, 2674-2676), and from these a very good approximation (better than 1%) when a and c are within an order of magnitude of each other can be found using:

$$K = 1 + r \ln(q) + s [\ln(q)]^2$$

Where $q = c/a$

For sedimentation in the direction parallel to c ; $r = -0.133$ and $s = 0.1$: (For completeness, sedimentation in the direction perpendicular to c ; $r = 0.068$ and $s = 0.097$ and for a rotational average; $r = 0.001$ and $s = 0.089$)

$D = (a^2c)^{(1/3)}$ is the equivalent volumetric diameter of the particle and D_0 the core diameter. In the limit that $a = D_0$, $q = (D/D_0)^3$. Then, with the spherical interpretation $D = D_0 + 2T$, in the limit $T \ll D_0$:

$$\ln(q) = 3\ln\left(\frac{D}{D_0}\right) = 3\ln\left(1 + \frac{2T}{D_0}\right) \approx \frac{6T}{D_0}$$

The basic DCS equation for sedimentation time can be rewritten to account for particle shape:

$$t = \frac{\alpha K}{(\rho_p - \rho_f)D^2}$$

Comparing a spherical interpretation, $K = 1$, with a prolate ellipsoid ($a = D_0$) moving parallel to the long axis, $K \approx 1 + (6Tr/D_0) = 1 - (0.8T/D_0)$, we obtain:

$$\frac{\Delta t_1}{t} = \frac{t(\text{ell}) - t(\text{sph})}{t(\text{sph})} \approx -\frac{0.8T}{D_0}$$

We have shown above in S.1, that in the case of a dense spherical particle, the change in sedimentation time after acquiring a thin spherical shell is:

$$\frac{\Delta t_2}{t} \approx \frac{2T}{D_0}$$

The combined effect of particle size and shape in this case would be a simple sum of the two changes, i.e.

$$\frac{\Delta t_1}{t} + \frac{\Delta t_2}{t} \approx \frac{1.2T}{D_0}$$

Which, if the sedimentation time was interpreted as a spherical shell with no shape change, would result in a 40% underestimation of the shell thickness for small shell thicknesses ($T \ll D_0$).

S.3

Example data:

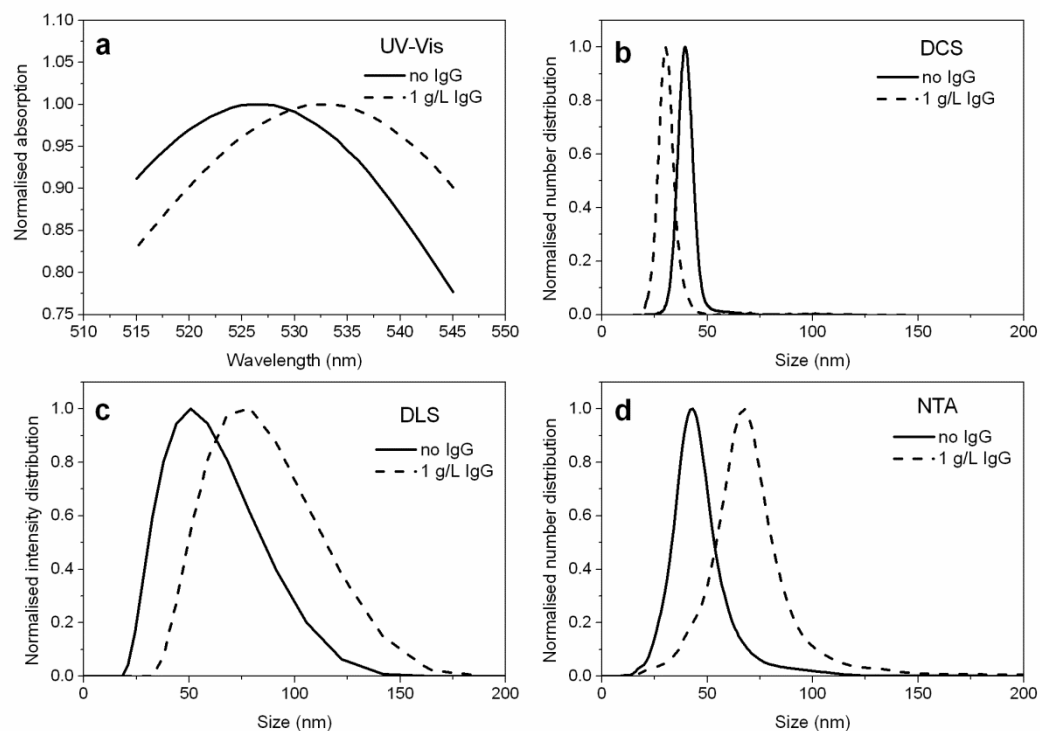


Figure S.3.1. Representative (a) UV-Vis, (b) DCS, (c) DLS and (d) DLS measurements performed on gold NPs having nominal size of 40 nm. Data is shown for NPs with no protein shell (continuous line) and with protein shell formed using $\rho_{\text{IgG}} = 1 \text{ g/L}$ (dashed line).

Table S.3.1. Measured parameters for gold NPs having nominal size of 40 nm with no protein shell (continuous line) and with protein shell formed using $\rho_{\text{IgG}} = 1 \text{ g/L}$. Errors are the standard deviation of several repeat measurements.

ρ_{IgG} (g/L)	λ_{max} (nm)	ζ -potential (mV)	DCS size* (nm)	DLS size (nm)	NTA size (nm)
-	526.48	-41.8 ± 1.5	35.9 ± 0.1	46.8 ± 0.5	43 ± 1
1	532.74	-22.0 ± 1.0	29.1 ± 0.2	71.0 ± 0.7	67 ± 1
	$\Delta\lambda_{\text{max}}$ (nm)	N/A	T_{DCS} (nm)	T_{DLS} (nm)	T_{NTA} (nm)
Derived values	6.26		14.0 ± 0.6	12.0 ± 0.9	12.0 ± 1.4

* Assuming $\rho_0 = 19.3 \text{ g cm}^{-3}$, from Eqn (1) in the paper, $T_{\text{DCS}}^*(0) = 5.33 \pm 0.14 \text{ nm}$ for bare particles and $T_{\text{DCS}}^*(1) = 19.34 \pm 0.58 \text{ nm}$ for 1 g/L IgG coated particles. Errors given here, and in the calculation of $T_{\text{DCS}} = T_{\text{DCS}}^*(1) - T_{\text{DCS}}^*(0)$ given in the table, do not account for uncertainty in any of the other parameters in Eqn (1). For example, an additional $\pm 1 \text{ nm}$ error arises from the uncertainty in D_0 , but this does not affect the precision of the results.